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Nonhelical supramolecular nanosystems

The present invention relates to a supramolecular nanosystem which comprises at least one essentially nonhelical oligomer (oligomer A) and one or more, identical or different, essentially nonhelical and mutually nonpairing oligomers with identical or different functional units (oligomer B), where oligomer A can undergo specific noncovalent pairing with oligomer B, and oligomer B can be determined through its monomers.

The miniaturization of technical components is now advancing into the area where sizes are of molecular magnitude. The production of miniaturized, integrated electronic circuits by conventional processes such as, for example, by photochemical treatment of a component is also determined by the particular chemical and physical properties of the materials used. In the nanometer region it is possible to utilize the discrete molecular or atomic-quantized material properties in order to produce novel components.

The material properties generated or influenced by nanostructuring are, in particular, optical or chiroptical properties, for example in Kerr cells and in the LEP technique; electrical properties, for example in semiconductors or conductors through constitution of conduction bands, positive holes, color centers or regions with tunneling currents which can be modulated; chemical catalytic properties such as, for example, with zeolites, metal cluster catalysis, constitution of reaction spaces; and physical surface and transport properties such as permeability, adhesion and compatibility with other materials or sensitive biological systems (biocompatibility).

In supramolecular chemistry, the nanomolecular properties described are utilized specifically to produce novel materials able to organize themselves in the form of pairing systems.

Pairing systems are supramolecular systems of noncovalent interaction which are distinguished by selectivity, stability and reversibility, and the properties thereof are preferably influenced thermodynamically, i.e., for

example, by temperature, pH and concentration. DNA and RNA play a fundamental role in this as carriers of genetic traits. However, it is also possible to use such pairing systems, for example because of their selective properties, as "molecular adhesive" for bringing together different metal clusters to give cluster assemblies with potentially novel properties [Mirkin, C. A. et al., Nature, 1996, 382, 607-9; Alivisatos, A. P. et al., Nature, 1996, 382, 609-11]. The pairing or hybridization properties of naturally occurring DNA have been used, for example, to bring about the pairing of metal clusters bound to DNA strands with a complementary DNA strand. This resulted in cluster assemblies with potentially novel material properties. Supramolecular nanosystems of these types can therefore be regarded as "molecular machines" or functional "molecular circuits".

Strong and thermodynamically controllable pairing systems are playing an increasingly important part for use in the area of nanotechnology for producing novel materials, diagnostic aids, therapeutic agents and microelectronic, photonic and optoelectronic components and for the controlled bringing together of molecular species to give supramolecular units.

However, DNA and RNA building blocks have the following disadvantages for producing pairing systems of these types:

a) The forces holding two strands together, in particular hydrogen bonds and stacking effects, are very small by their nature. Such duplexes therefore have low stability. This can easily be established by recording a so-called transition plot and determining the transition point. Consequently, relatively long single strands are needed to produce pairing systems, which results in the content of the pairing system predominating in the supramolecular unit, i.e. the "nucleotide load" is large.

b) The selectivity decreases owing to the formation of *Hoogsteen* pairings which are a possible alternative to *Watson-Crick* pairings. This is often associated with parallel duplexes or irreversible pairing processes.

c) The high flexibility of the sugar-phosphate backbone leads to formation of helical conformations, which means that the spatial arrangement in supramolecular units can be controlled less well.

d) A possible interference with the genetic material of biological systems cannot be precluded if the supramolecular units are employed in a biological system, i.e. there is no orthogonality of the pairing process.

This means that the use of DNA or RNA building blocks for example in two- and three-dimensional supramolecular structures with complementary binding (see, for example, WO96/13522) in a physiological medium is possible only with difficulty, in particular in view of item d).

It was therefore an object of the present invention to find a system which avoids one or more of the described disadvantages as far as possible.

It has now been found, surprisingly, that essentially nonhelical supramolecular nanosystems represent particularly advantageous building blocks.

The present invention therefore relates firstly to a supramolecular nanosystem which comprises at least one essentially nonhelical oligomer (oligomer A) and one or more, identical or different, essentially nonhelical and mutually nonpairing oligomers with identical or different functional units (oligomer B), where oligomer A can undergo specific noncovalent pairing with oligomer B, and oligomer B can be determined through its monomers.

Noncovalent pairing means for the purpose of the present invention an association of oligomer A with oligomer B via noncovalent interactions such as, for example, hydrogen bonds, salt links, stacking, metal ligands, charge transfer complexes and hydrophobic interactions.

The meaning of "can be determined" for the purpose of the present invention is that the functional unit is addressed, i.e. encoded, by the oligomer. The code is defined by the previously established sequence and nature of the monomers. This can be, for example, a particular nucleotide sequence.

The nature and sequence of the monomers of oligomer B determines the nature and sequence of the monomers of oligomer A. In the case of nucleotides, these are the mutually complementary nucleotides (see, for example, Figure 2).

In a particular embodiment, oligomer A is able to pair both with oligomer B and with itself in the form of a hairpin loop. Depending on the external conditions, this can make it possible easily to induce and determine structural changes macroscopically (see, for example, Figure 4). For example, it is possible to cause structural changes in the molecular nanosystem according to the invention by changing the equilibrium conditions such as, for example, concentration of oligomer B, salt concentration, pH, pressure and/or temperature. It is also possible by adjusting particular equilibrium conditions to bring about pairing or unpairing of various regions so that initially remote residues in the molecule can be brought reversibly into direct vicinity (so-called nanotransport).

In a preferred embodiment, the essentially nonhelical oligomer is a pentopyranosyl-nucleic acid, in particular a ribo-, arabino-, lyxo- and/or xylo-pyranosyl-nucleic acid, preferably a ribopyranosyl-nucleic acid also called pyranosyl-RNA (p-RNA).

The p-RNA as an example of a pentopyranosyl-nucleic acid is a nucleic acid which, in place of the ribofuranose of RNA, contains the ribopyranose as sugar building block and therefore forms exclusively *Watson-Crick*-paired, antiparallel, reversibly "melting", *quasi*-linear and stable duplexes. In addition there are also homochiral p-RNA strands of opposite handedness which likewise pair in a controllable manner and are not strictly helical in the duplex formed. This specificity, which is valuable for constructing supramolecular units, is connected with the relatively low flexibility of the ribopyranose phosphate backbone and with the large inclination of the base plane relative to the strand axis and the tendency, resulting therefrom, to intercatenary base stacking in the resulting duplex, and can be attributed to the participation of a 2',4'-cis-disubstituted ribopyranose ring in the structure of the backbone. Because of the high selectivity and stability and the formation of strictly planar linear duplex strands, the pentopyranosyl-nucleic acid and, preferably, the p-RNA is particularly preferred for the present invention. All residues bound in the same way to the pentopyranosyl strand are located on the same side of the duplex, which is particularly advantageous. Pentopyranosyl-nucleic acids can be prepared, for example, as described by Eschenmoser et al. (Helv. Chim. Acta 1993, 76, 2161; Helv. Chim Acta 1995, 78, 1621; Angew. Chem. 1996, 108, 1619-1623) and generally have the D or L configuration.

The natural model for the preparation of the supramolecular nanosystem according to the invention is the decoding of amino acids for protein synthesis by the relevant base triplets as anticodon (see Figure 1). In analogy to this, according to the present invention identical or different functional units are bound to an oligomer of a defined structure. For example, a pentopyranosyl-oligonucleotide which is modified at the 3' and/or 5' end with free sulfhydryl groups is bound to monomaleimido-derivatized gold particles (in analogy to Alivisatos, A.P. et al. (1996), supra). The oligomer modified in this way (called oligomer B) is brought into contact for pairing with an oligomer A complementary thereto, so that the supramolecular nanosystem according to the invention can form. The duplex strands which form are generally in an essentially planar linear form, which is particularly advantageous.

Oligomer A is generally longer than oligomer B. A particularly preferred length of oligomer A is from about 10 about 500, preferably from about 10 to about 100, monomer units. Oligomer B is generally about 4 to about 50, preferably about 4 to 25, in particular about 4 to about 15, especially about 4 to about 8, monomer units long.

In another embodiment, the pentopyranosyl part of the pentopyranosyl-nucleic acid can be modified in the form of a thiophosphate, alkylated phosphate, phosphonate and/or amide (see, for example, Uhlmann E. and Peyman A. (1990) Chemical Reviews, 90, 543-584, No. 4). In another embodiment of the present invention, one of the canonical nucleobases adenosine, guanosine, cytosine, thymidine and/or uracil or else isoguanosine, isocytosine, 2,6-diaminopurine and/or xanthine is used for coding the oligomers. In the last-mentioned cases, the complementary bases are present in the form of isoguanine/isocytosine or 2,6-diaminopurine/xanthine pairs. Otherwise, generally adenosine pairs with thymidine or uracil and guanosine pairs with cytosine.

In another embodiment, the noncovalent pairing between oligomer A and oligomer B can take place via a chelating agent. In this case for example the nucleobases of a pentopyranosyl-nucleic acid are replaced by the chelating agent. Examples of chelating agents suitable for this purpose are those derived from pyrazolylpyridine or pyridoquinazoline. In the presence of a metal ion, for example Cu^{2+} or Ni^{2+}, complexation takes place, and thus specific pairing between the two oligomers (see Figure 3).

The functional unit generally suitable for oligomer B is a metal, preferably a metal cluster, in particular a noble metal, especially gold, silver and/or platinum. Semiconductor compounds are also suitable, such as, for example, cadmium selenide and/or cadmium sulfide. Also suitable as functional unit is a peptide which can be bound to the oligomer via a suitable linker, for example N-phthaloylaminoethyluracil or N-phthaloyltryptamine. Another functional unit is, for example, a redox center, i.e. an electron donor or acceptor, for example a quinone or hydroquinone. Fluorescent labels, for example fluorophores, and/or chromophores such as, for example, benzoquinones or azobenzenes are also suitable. Other functional units may represent a chelating agent which is preferably derived from anthocyans, polyoxycarboxylic acids, polyamines, dimethylglyoxime, ethylenediaminetetraacetic acid and/or nitrilotriacetic acid, or else conducting oligomers such as, for example, conjugated alkyne-alkene-aromatic compounds. The linkage of an oligomer to a functional unit which results in the oligomer B can in general be carried out using linkers known to the skilled worker (see, for example, Mirkin C.A. et al. (1996), Nature, 382, 607-609; Alivisatos, A.P. et al. (1996), supra; Dawson, P.E. et al. (1994), S.B.H. Kent Science, 30, 776-779; Liu C.-F. et al. (1996), 116, 4149-4153) or using purchasable base and amidite linkers (Wei Z. et al. Bioconjugate Chem. (1994), 5, 468-474; Liu C.-F. et al. (1991), Proc. Natl. Acad. Sci. USA, 91, 6584-6588). The oligonucleotides themselves can be prepared, for example, automatically in an oligonucleotide synthesizer.

In another embodiment, oligomer A can be linked, i.e. fixed, with oligomer B after the association. Chemical fixation is preferred, for example a covalent crosslinking, metathesis, Heck coupling, Michael addition of thiols and/or oxidative formation of disulfide bridges. It is particularly preferred for the supramolecular nanosystem according to the invention to be absorbed on a solid phase, for example a so-called wafer or support.

Examples of suitable support materials are ceramics, metal, in particular noble metal such as gold, silver or platinum, glasses, plastics, crystalline materials or thin layers of the support, in particular of said materials, or (bio)molecular filaments such as cellulose or scleroproteins.

Attachment to the support generally takes place covalently, quasi-covalently, supramolecularly or physically such as magnetically (Shepard, A.R. (1997) Nucleic Acids Res., 25, 3183-3185, No. 15), in an electric field or through a molecular sieve. For example, oligomer A can be either synthesized directly in position on the support or be linked to particular positions on the support. Examples are conjugation and attachment methods via periodate oxidation and reductive amination of the Schiff's base, N-hydroxysuccinimide esters of, preferably, dicarboxylic acid linker, ethylenediaminephosphoramidate linker, mercapto, iodoacetyl or maleimido methods and/or covalent or noncovalent biotin linker methods.

Another embodiment of the present invention is a library comprising a plurality of different supramolecular nanosystems according to the invention. It is particularly advantageous for the library to be constructed by combinatorial methods. A library constructed by combinatorial methods is suitable, for example, for screening for properties by pairing a (sub)library prepared randomly or by combinatorial deconvolution techniques with the complementary oligonucleotide (see, for example, Wilson-Lingardo (1996) J. med. Chem., 39, 2720-2726).

In the case where the functional unit of oligomer B is, for example, a metal cluster, a library produced combinatorially is particularly suitable for searching for catalysts. For this purpose, for example, oligomer A is synthesized combinatorially and paired with a plurality of different oligomers B with different metal clusters as functional units. This results in a so-called cluster library whose diversity correlates directly with that of oligomer A. Suitable and preferred for this are sublibrary routines which allow simple identification of the active species, such as, for example, positional scanning or orthogonal libraries. The cluster library can subsequently be examined for its homogeneous catalytic properties for example in water for vinyl acetate monomer catalysis.

It is advantageous in general and particularly for producing libraries if the pentopyranosyl-nucleic acid has a relatively high cytosine and guanosine content because, owing to the higher enthalpy of binding of this nucleotide pair compared with adenosine and thymidine, shorter oligonucleotides can be used, which makes it possible to reduce the "nucleotide load" in the supramolecular nanosystem according to the invention.

Replacement of the nucleobases by one or more identical or different chelating agents as described in detail above is able to reduce the "nucleotide load" further. This results in formation of one-center complexes which form linear, nonhelical, oligomeric metal complexes. Because of the arrangement like branches in one plane, the pairing process can react optimally to the size of the different metal centers. The duplexes formed in this way generally have an inclined, but nonhelical, repetitive structure, coordinates specific metal centers depending on the choice of the ligand, and makes metal-metal interactions or desired defects possible along the duplex axis. This makes it possible to produce in a controlled manner metal sequences which represent a novel nanoalloying kit for producing so-called "nanowires".

It is also possible with the supramolecular nanosystems according to the invention described above for example to spatially position different metal clusters on the supramolecular plane with a view to constructing electronic switching patterns (see, for example, Andres R.P. (1996) Science, 272, 1323-1325). It is also possible to construct so-called cluster lattices with rod-shaped dithiols, which show good stability (see, for example, Andres R.P. et al. (1996) Science, 273, 1690-1693; Schiffrin D.J. et al. (1995) Adv. Mat., 7, 795-797).

The described supramolecular nanosystem according to the invention has particularly high stability and selectivity and is particularly suitable for self-organization. In addition, it has controllable topicity and the aggregation or self-organization can be dynamically influenced particularly well.

Areas of application are therefore in particular the production of electronic components such as, for example, information storage media, diagnostic probes or photoelectronic components; catalysts; semiconductors; photochemical units; biocompatible materials or units or functional microprostheses.

The following figures and examples are intended to illustrate the invention in detail without restricting it thereto.

Description of the figures:

Fig. 1: Diagrammatic representation of the natural base pairing in peptide synthesis

Fig. 2: Diagrammatic representation of a supramolecular nanosystem according to the invention with the nucleobases adenosine (A) and thymidine (T) and various functional units designated XI to XI (coding units).

Fig. 3: Diagrammatic representation of a one-center chelate complex with a pyrido[3,2-h]quinazolin-2(1)-one as chelating agent.

Fig. 4: Diagrammatic representation of an equilibrium reaction between a hairpin loop and a duplex.

Fig. 5: Section of an x-ray structure analysis of a nickel chelate-ribopyranose-pyrazolylpyridine complex

Examples

1. Preparation of a gold cluster-pyranosyl-RNA

Pyranosyl-RNA was prepared by the method of Eschenmoser et al. (supra) by a phosphoramidite synthesis. Gold clusters were bound to one strand as described in Mirkin C.A. et al. (1996), supra. The complementary strands were paired in a buffer solution (1 M NaCl, 10 mM Tris-HCl, pH 7) at 0°C (see Fig. 2).

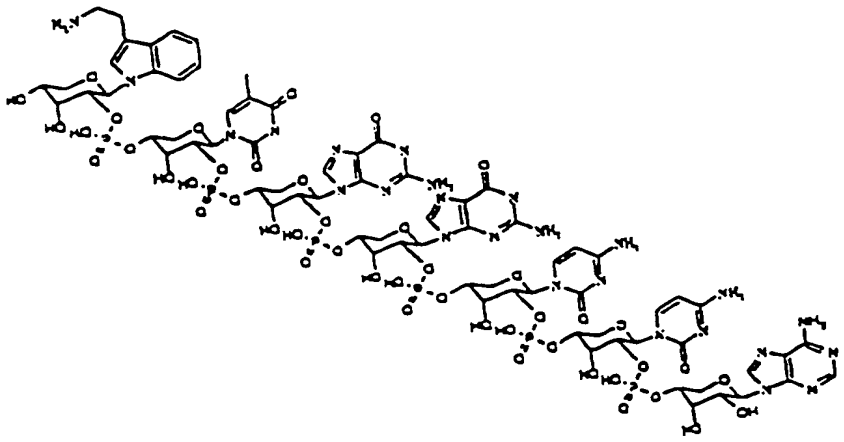
2. Preparation of a self-complementary oligonucleotide with the sequence ITGGCCA

The automatic solid-phase synthesis of the oligonucleotide with the sequence ITGGCCA was carried out as described by Pitsch S. et al. (1993) Helv. Chim. Acta 78, 1621-1635. The average yield in an Eppendorf Ecosyn D300+ automatic synthesizer was 93.2%. The coupling times were 45 min, the oxidation time was 2 min and the detritylation times were 7 min with dichloroacetic acid flowing through. After the synthesis, the oligonucleotide was deprotected with tetrakistriphenylphosphinepalladium (20 mg for a 1 µmol batch of support) with the addition of 20 mg of diethylammonium bicarbonate and 20 mg of triphenylphosphine at room temperature for five hours, then washed with acetone and water and treated with fresh aqueous sodium dithiocarbamate solution for 45 min. The

product was then cleaved off by a 24% strength hydrazine hydrate solution at 4°C, rotating for 24 hours. Salts were removed in a reverse phase Sep-Pak cartridge, and purification was carried out by RP-HPLC (RP-18, water/acetonitrile gradient, pH 7). Salts were then removed again, and lyophilization resulted in the "trityl-on" product. This was deprotected with 80% strength formic acid, evaporated, taken up in 10 ml of water, extracted with dichloromethane and again purified by HPLC. 8 OD of the required product were obtained.

Examination by mass spectrometry gave the following result:

Samples: LX626-1: MS-No.: 970523
Objective: Characterization of the sample by mass spectrometry



Mass spectrometer: TSQ 700 (Finnigan/MAT)
Measuring conditions: MS; injection pump
Ionization: Electrospray ionization (ESI)
Results: The mass spectrum shows a molecular mass M = 2242

3. Molecular nanokinematics

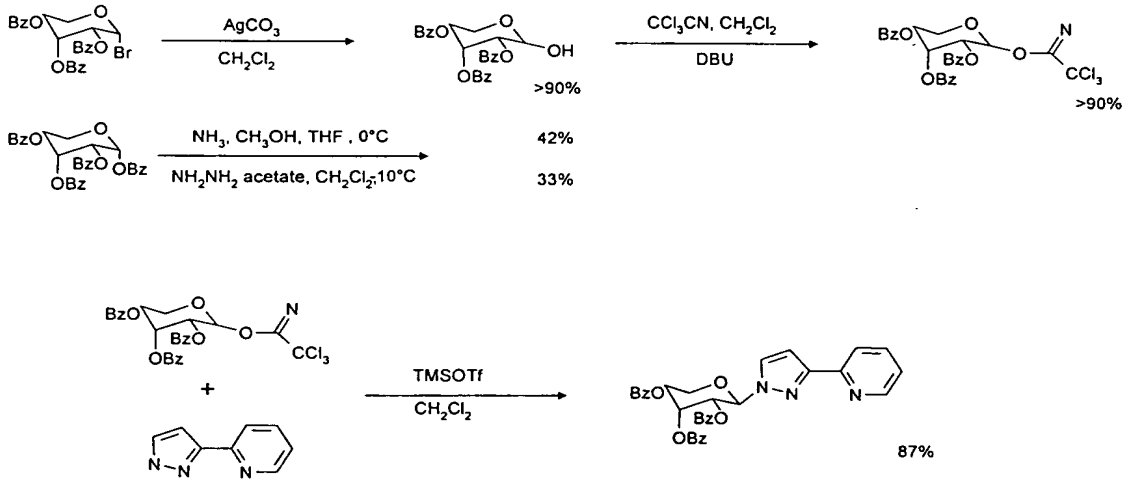
The phosphoramidite method was used to synthesize a pyranosyl-RNA strand which was partly self-complementary as hairpin and had 4' and 5' linker ends with the sequence linker-pr-GCGA$_5$CGC-linker, and the linker ends were linked to maleimido-gold clusters as described by Alivisatos, A.P. et al. (1996), supra. Then the pairing of 10 mM product to the hairpin was detected by spectroscopy in standard buffer (0.15 M NaCl or 1 M NaCl, 10 mM Tris HCl, pH 7). Addition of one equivalent of the

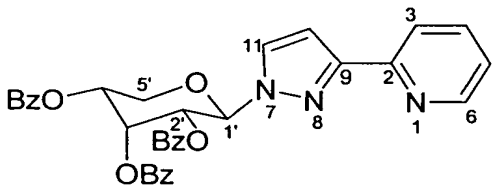
complementary strand pr-G(T_5)C proved by spectroscopy the opening of the hairpin and the separating of the gold clusters. The hairpin structure was restored simply by diluting the solution. It is possible in this way to expose a substrate to different reaction centers macroscopically in a controlled manner via the dilution (see Fig. 4).

4. Synthesis of a p-RNA-pyridylpyrazole ligand as monomer for oligomeric ligands

The following reaction scheme shows the preparation of the 2-[1-(2',3',4'-tri-O-benzoyl-1'ß-ribopyranosyl)-3-pyrazolyl]pyridine:

Preparation of
2-[1-(2',3',4'-tri-O-benzoyl-1'β-ribopyranosyl)-3-pyrazolyl]pyridine





0.50 g (3.44 mmol) of 2-[3(5)-pyrazolyl]pyridine was dissolved in 30 ml of CH_2Cl_2 and cooled to -15°C. 2.30 g of 2',3',4'-tri-O-benzoyl-1'-D-ribopyranosyl trichloroacetimidate in 15 ml of CH_2Cl_2 were slowly added dropwise. The solution became pale yellow. Then 0.8 ml (1.2 equivalents)

of TMSOTf in 15 ml of CH_2Cl_2 was added dropwise at -15°C over the course of 15 minutes. The solution became cloudy, and a white precipitate formed. The solution was then stirred at between -10°C and +5°C for 5 hours. The solution was then filtered and concentrated. The product was purified by flash chromatography on silica gel (CH_2Cl_2/acetone : 95/5): 1.77 g (3 mmol, 87%) of product.

Rf : 0.47 (CH_2Cl_2/acetone : 9/1)

Melting point : 91 - 93°C (CH_2Cl_2/isohexane).

UV (CH_3CN) **:** λ = 202 nm ε = 21522
λ = 230 nm ε = 35826
λ = 274 nm ε = 7696

^{1}H NMR : δ (ppm) = 8.60 (dm, J=4.8 Hz, 1H, 6-H); 8.07 (d, J=8.5 Hz, 2H, 2*o-benz.-2'); 7.98 (d, J=7.8 Hz, 1H, H-3); 7.93 (d, J=8.3 Hz, 2H, 2*o-benz.-3' or 4'); 7.82 (d, J=8.3 Hz, 2H, 2*o-benz.-3' or 4'); 7.77 (d, J=2.6 Hz, 1H, H-11); 7.70 (td, J=7.6 and 1.8 Hz, 1H, H-4); 7.62 (t, J=7.5 Hz, 1H, p-benz.-4'); 7.52 (t, J=7.5 Hz, 1H, p-benz.-3'); 7.48 (t, J=7.5 Hz, 2H, 2*m-benz.-4'); 7.46 (t, J=7.5 Hz, 1H, p-benz.-2'); 7.34 (t, J=7.8 Hz, 2H, 2*m-benz.-3'); 7.25 (t, J=7.7 Hz, 2H, 2*m-benz.-2'); 7.19 (ddd, J=7.6, 4.8 and 1.8 Hz, 1H, H-5); 6.99 (d, J=2.6 Hz, 1H, H-10); 6.49 (t, J=3.1 Hz, 1H, H-3'); 6.17 (d, J=6.8 Hz, 1H, H-1'); 6.11 (dd, J=6.8 and 3.1 Hz, 1H, H-2'); 5.69 (m; 1H, H-4'); 4.32 (dd, J=11.2 and 8.2 Hz, 1H, H-5'); 4.28 (dd, J=11.2 and 8.2 Hz, 1H, H-5').

The signals were assigned with the aid of a **^{1}H,^{1}H-*COSY*** spectrum.

^{13}C NMR : δ (ppm) = 165.23 (CO-4'); 165.17 (CO-3'); 164.88 (CO-2'); 152.85 (C-2); 151.51(C-9); 149.16 (C-6); 136.62 (C-4); 133.50 (C-p-benz.-4'); 133.35 (C-p-benz.-3'); 133.32 (C-p-benz.-2'); 130.44 (C-11); 129.79 (2*C-o-benz.-4'); 129.78 (2*C-o-benz.-3'); 129.73 (2*C-o-benz.-2'); 129.37 (C-i-benz.-4'); 129.11 (C-i-benz.-3'); 128.78 (C-i-benz.-2'); 128.61(2*C-m-benz.-4'); 128.37 (2*C-m-benz.-3'); 128.24 (2*C-m-benz.-2'); 122.75 (C-5); 120.47 (C-3); 105.97 (C-10); 85.42 (C-1'); 68.57 (2C-2' and 3'); 66.97 (C-4'); 63.85 (C-5').

The signals were assigned with the aid of a $^{1}H,^{13}C$-*COSY* spectrum.

NOESY : NOE between H-11 and H-1', H-2' : Proof of linkage of C-1' to N-7

MS : Electrospray ionization (ESI) $[MH^{+}]= 590$

$C_{34}\ H_{27}\ N_3\ O_7$ M= 589

X-ray structure analysis of crystals of the monomer proved the correct glycosidic linkage after recrystallization from CH_2Cl_2/isohexane. The benzoylated monomer showed the required complexing properties (UV, NMR) after treatment with alcoholic nickel(II) chloride hydrate solution (reflux). This result was confirmed by an X-ray structure analysis of the nickel chelate-ribopyranose-pyrazolylpyridine complex (Fig. 5)

This showed the replacement of the nucleobase capable of pairing by a strong nitrogen back-bonding ligand. The monomer prepared and protected in this way in the form of the D enantiomer can be converted as described above into the protected p-RNA-phosphoramidite.